were lowered, possibly mimicking the changes in disease where further hypoxia and acidosis are known features. Addition of IL-1β increased ROS levels in every condition, (except 0% O_2) possibly through inducing a respiratory burst. A decrease in GSH appears responsible for the decreases in the GSH: GSSG ratio seen in hypoxic and/or acidic conditions with or without IL-1β. This work demonstrates the importance of studying oxygen and pH on mitochondrial function in chondrocytes. The mechanisms behind this oxygen and pH-sensitivity require further characterisation.

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CHANGES IN THE INTEGRINS EXPRESSION ARE RELATED WITH THE LOSS OF EXTRACELLULAR MATRIX DURING THE OSTEOARTHRITIS PATHOGENESIS.

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Purpose: The aim of this study was to identify changes in the integrins expression from chondrocytes of the three zones of the cartilage and their possible role during the Osteoarthritis (OA) pathogenesis in an animal model.
Methods: The experimentally OA-induced model was accomplished by unilateral knee menisectomy and post-surgery training; normal rats were used as a control. Animals were sacrificed by CO2 overdose and right femoral condyles were removed and processed for electron microscopy (TEM) and Immunohistochemistry (IHC). Changes at ultrastructural level were observed by TEM at early stages of OA. In addition, the expression of integrins α2 and α5, as well as collagen I, collagen II and the caspase 3 active (C3A) were identified in cartilage at 1, 3, 5, 10, 20 and 45 training days (td) by IHC. At the same time, as a complementary method was identified the loss of proteoglycans (PG) through safranin O staining.
Results: Since early stages of OA, chondrocytes undergo changes at ultrastructural level as well as in their relationship with the extracellular matrix (ECM); these changes started in the superficial (SZ) and middle zones (MZ). In addition, integrins α2, α5 and collagen I were increased during the OA pathogenesis, showing their highest expression at 45 td in the SZ and MZ; however, collagen II and proteoglycans showed a tendency to decrease at late stages of OA. Furthermore the cell death was increased in late stages of OA mostly in the SZ and deep (DZ) zones.
Conclusions: The loss of ECM and death of chondrocytes are the central features during the OA pathogenesis. Our results suggest that in early stages of OA, chondrocytes changes present at ultrastructural and morphological levels, could be are related with their increased synthetic activity induced as a consequence of the mechanical damage. However, at late stages of OA, the loss of ECM (PG and collagen II) induces the increased expression of integrins; probably as a result of the inefficient ECM remodeling (synthesis of collagen I) and with the aim to avoid the cell death by the loss of survival signals. In addition, during OA pathogenesis, the presence of ECM fragments shows a proteolityc activity on the cartilage, inducing changes in the normal integrins signaling, which increase the OA severity.

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DIFFERENTIAL IMPACT OF GLUCOSAMINE SULFATE AND CUIVRAMINE ON THE IL-1β STIMULATED C-20/A4 CHONDROCYTE CELL LINE, *IN VITRO*.

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Purpose: The pathogenesis of primary osteoarthritis involves an imbalance between anabolic and catabolic pathways in chondrocytes where the reactive oxygen species (ROS) could play a central role. The expression of matrix metalloproteinases (MMPs), chondrocyte hypertrophy and apoptosis are the main features of the pathology. NADPH oxidase Nox4 is one of the 7 ROS generating Nox members expressed in human. It was shown to be expressed in human primary chondrocytes. Nox4 displays a constitutive NADPH oxidase activity that was previously reported to modulate proMMP1 expression and apoptosis of the C-20/A4 cell line. This pathway is down regulated by heme oxygenase-1 (HO-1). Glucosamine sulfate (GS), a basic structural element that composes cartilage proteoglycans is also a dietary supplement approved as a symptomatic slow-acting drug for osteoarthritis (SYSADOA). However, impact of GS on structural features of OA is relatively modest. To go further, Cuivramine (CA), a new dietary supplement containing GS (78.9%), copper sulfate (0.105%) and ginger root extract (5.26%) has been developed.
The aim of the study is to compare *in vitro* the effects of CA and GS on IL-1β stimulated C-20/A4 chondrocytes. Impact on ROS production (1), related MMPs expression (2), chondrocyte apoptosis (3) and mechanisms of action (4) has been investigated.
Methods: The antioxidant effects of GS and CA were investigated on HEK 293 TRex cells, a reproducible and reliable cell model to study Nox4. MMP expression and apoptosis were assessed on the human C-20/A4 chondrocyte cell line. The cells were pre-treated with 100 or 500μg/ml CA or GS during 96h. Then, they were stimulated by 2ng/ml IL-1β for 24h to assess the secretion of ADAMTS5, proMMP1 and proMMP13 or 96h to evaluate caspase 3 activation and viability. The expression of the antioxidant protein HO-1 was assessed by Western Blot.
Results: No direct antioxidant effects of CA and GS have been reported on the HEK 293 TRex cell line. However, the ROS production was significantly decreased (30%) after 96h pre-incubation with 500μg/ml CA. The proMMP1 expression was shown to be modulated by Nox4 derived ROS in C-20/A4 chondrocytes. The results have shown a significant decrease in proMMP1 expression (40%) in CA treated chondrocytes but not after GS treatment. This effect was dependant on ginger root and copper sulfate. Furthermore, ADAMTS5 expression was markedly decreased by GS and CA but not by ginger root and copper sulfate. On the other hand, there was no effect on proMMP13 expression. Moreover, results reported a significant decrease in the IL-1β induced caspase 3 activation in presence of GS and CA. Our data suggest that molecular mechanisms could involve HO-1.
Conclusions: In this study we provided experimental evidence *in vitro* that glucosamine sulfate decreases ADAMTS5 expression and apoptosis in the IL-1β stimulated C-20/A4 chondrocytes. In addition, ginger root and copper sulfate decrease the Nox4 regulated proMMP1 expression. These findings emphasize *in vitro* the potential beneficial effects of Cuivramine in osteoarthritis.

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THE EXPRESSIONS OF TYPEII COLLAGEN IS REGULATED BY PTEN IN HUMAN CHODROCYTES.

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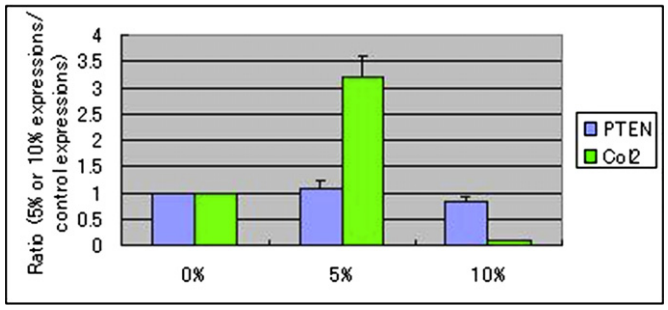
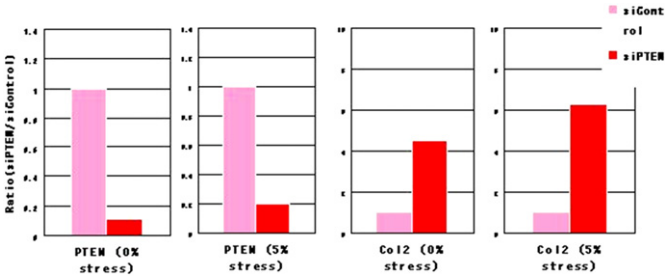
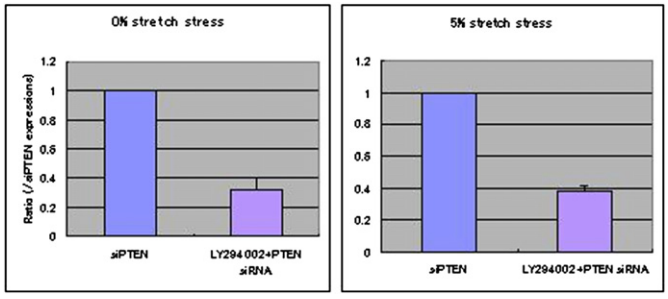
Purpose: Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) was identified as an important tumor suppressor gene. PTEN is second most frequently mutated gene in human cancer after p53. The function of PTEN is one of negative regulator of phosphoinositol-3-kinase (PI3K) signaling. PI3K pathway is critical for cell survival, differentiation and matrix synthesis. We have reported that apoptosis by shear stress in chondrocytes was dependent on p53, but the functions of PTEN in chondrocytes are still unknown. Therefore, we investigated the function of PTEN in chondrocytes.
Methods: Normal human chondrocytes were cultured on silicone chambers (STREX, Osaka, Japan), and stretched at 5% stress for 6 hours by pulse-motor-driven stretch machine (STREX). In order to inhibit the function of PTEN, PTEN siRNA was transfected to chondrocytes by lipofection method. After transfection of PTEN siRNA, chondrocytes were applied 5% or 10% stretch stress for 24 hours.
Further, chondrocytes were transfected with PTEN siRNA and treated with PI3K specific inhibitor (LY294002) and then, chondrocytes were applied at 5% stretch stress.
The expressions of PTEN and type II collagen (Col2a1) mRNA were analyzed by real-time PCR.
Results: The expression levels of PTEN were not changed by stretch stress, but the expression levels of Col2a1 were increased by 5% stretch stress and were decreased by 10% stretch stress (Figure1).

After transfection of PTEN siRNA, the expressions of Col2a1 were increased and were much more increased when 5% stretch stress was applied in comparsion with control siRNA (Figure2).
The expressions of Col2a1 were inhibited after exposure to PTEN siRNA with LY294002 (Figure3).
Conclusions: PTEN is one of the most important tumor suppressor like p53. We have reported that p53 was increased by shear stress, but PTEN was not increased. The function of PTEN depends on its abundant expression. However, p53 acts emergently when DNA is damaged. The role of PTEN in human chondrocytes might be different from p53.
Col2a1 expression was reported to be regulated via PI3K/Akt pathway. We confirmed that the expressions of Col2a1 were decreased with attenuation of Akt phosphorylation when PI3K activity was blocked by LY294002. Down-regulation of PTEN expression increased Col2a1 expression with activation of Akt phosphorylation. Therefore, PTEN regulates the expressions of Col2a1 by inhibition of Akt phosphorylation.
In conclusion, PTEN regulates the expressions of typeII collagen in human chondrocytes. So, PTEN may also have an important role in cartilage repair via PI3K/Akt pathway in human chondrocytes.







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O-GLCNAC PROTEIN MODIFICATION STIMULATES CHONDROGENESIS IN VITRO AND CHONDROCYTE HYPERTROPHY IN MOUSE

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Purpose: Chondrocyte differentiation that allows endochondral ossification sequentially includes cell proliferation, extracellular matrix synthesis, cellular hypertrophy, matrix mineralization, vascular invasion and eventually apoptosis, allowing cartilage remodeling into bone. Most of these processes have been also associated to the development of osteoarthritis. Insulin ameliorates impaired bone growth and impaired bone healing both in vitro and in vivo, and is able to induce chondrocyte hypertrophy and growth plate chondrogenesis, although the specific molecular mechanisms are mostly unknown. The addition of O-linked N-Acetylglucosamine (O-GlcNAc) to cytoplasmic and nuclear proteins is a post-translational modification implicated in the regulation of a variety of transduction pathways. Our aim was to investigate whether insulin-induced chondrocyte hypertrophy occurs through a modification in the amount of O-GlcNAc glycosylated proteins and in the expression of the key enzymes of this pathway: O-GlcNAc-transferase (OGT) and O-GlcNAcase (OGA). Furthermore, we studied if O-GlcNAc-accumulation *per se* was able to induce pre-hypertrophic chondrocyte differentiation both in vitro and in vivo.
Methods: Pre-chondrogenic ATDC5 cells were cultured in 5% FBS DMEM/F12. Cell differentiation was induced with 10 μg/ml insulin and studied during 24 days. To induce the accumulation of O-GlcNAc modified proteins, we employed the specific OGA inhibitor Thiamet-G (TG). The gene expression of the differentiation markers collagen X (ColX), PTH receptor (PTHR), Indian hedgehog protein (IHH), Runx-2, and alkaline phosphatase (AP) was studied by real time PCR experiments employing TaqMan probes, while the activity of matrix-metalloproteinase (MMP)-2, -3 and -9 were measured by zymography. The accumulation of O-GlcNAc modified proteins, and the presence of OGT, OGA, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 were studied by western-blot techniques. The effect of the in vivo accumulation of O-GlcNAc proteins in the tibial growth plate was studied in 23-day old C57/bl mice that received 20mg/kg TG during 15 days. Then, animals were euthanized and tibias were fixed and embedded in paraffin for histological studies.
Results: Insulin-induced differentiation paralleled with a gradual increase in the accumulation of O-GlcNac modified proteins that was appreciable since day 3 of differentiation and peaked around day 7-9, together with an increase in the expression of OGT and OGA. These increases occurred previously to any modification in the gene expression of differentiation markers, such as ColX, IHH, PTHR1, Runx-2 or AP. Furthermore, O-GlcNAc accumulation per se induced by OGA inhibition, in the absence of insulin, was able to induce ATDC5 differentiation measured by an increase in the expression of differentiation markers. TG treated ATDC5 cells also induced an increase in the activity of MMP-1,-2 and -9, similar to that evoked by insulin. In addition, TG induced the activation of p-ERK, p-JNK and p-p38 in a similar extent to that observed for insulin. The administration of TG to C57/bl mice induced an accumulation of O-GlcNAc modified proteins in different locations, such as brain, liver, muscle and knee joint. Furthermore, TG induced a significant expansion in the growth plate height and in the hypertrophic zone height, in comparison to untreated mice, without a significant modification of body weight gain.
Conclusions: TG markedly induced the expression of chondrogenic markers in ATDC5 cells, as well as activated ERK, JNK and p38. TG also increased chondrogenic differentiation in vivo. Taken together our results show that O-GlcNAc glycosylation has chondromodulating activity, opening new therapeutic targets both in the alterations in endochondral ossification and in osteoarthritis.

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DIFFERENTIAL EFFECTS OF CYCLOOXYGENASE-1 AND -2 SPECIFIC NSAIDS ON CHONDROGENIC DIFFERENTIATION

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Purpose: NSAIDs are clinically used to relieve pain and decrease inflammatory reactions by inhibition of cyclooxygenase (COX)- catalyzed prostaglandin (PG) synthesis. As PGs are important fatty acid mediators in bone and cartilage homeostasis, they thereby may provide a novel opportunity to influence cartilage regeneration. In this study we aimed to define how different COX-1 or COX-2 specific NSAIDs influence chondrocyte extracellular matrix formation.
Methods: ATDC5 cells were differentiated in the presence of different COX-1 or COX-2 specific inhibitors. COX-1 or COX-2 specificity of the